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EVALUATION OF ANTICANCERC POTENTIAL OF SELECTED PLANTS OF CUCURBITACEA

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EVALUATION OF ANTICANCERC POTENTIAL OF SELECTED PLANTS OF CUCURBITACEA

1. Introduction:

Cancer is a severe metabolic syndrome and is one of the leading cause of death regardless of developments in the tools of disease diagnosis, treatment and prevention measures (Qin et al., 2017). Cancer is one of the principal causes of mortality around the globe and the number of cases are constantly increasing estimated to be 21 million by 2030 (Siegel et al., 2016). This uncontrolled proliferation of a normal cell which produces genetic instabilities and alterations accumulates within cells and tissues which transforms normal cell into a malignant cell. These genetic instabilities include mutations in DNA repair genes (*p21, p22, p27, p51, p53*), tumor suppressor genes (*p53, NF1, NF2, RB* and biological breaks), oncogenes (*MYC, RAF, Bcl-2, RAS* (biological accelerators) and genes involve in cell growth metabolism. Both external factors (radiations, smoking, tobacco, pollutants in drinking water, food, air, chemicals, certain metals and infectious agents) and internal factors (genetic mutations, body immune system and hormonal disorders) can cause cancer (Krishnamurthi, 2000) There are several types of cancer in human being; among these the lung cancer is reported the top listed in male followed by breast cancer in female. Previously, around 10.9 million new cancer cases, 24.6 million persons living with cancer, 6.7 million deaths reported around the world each year. Based on World Health Organization data, above 14.1 million new cancer cases and 8.2 million deaths were mentioned globally in the year 2012 and over 70% new cancer cases has been estimated during the next twenty years (Bray et al., 2018). Nearly, 80% of the world's population depends on traditional medicines and more than 60 % of clinically approved anticancer drugs are derivatives of these medicinal plants (Cragg et al., 2006).

Role of herbal medicine as Complementary Therapy in the Management of cancer treatment:

Plants are being used as indigenous cure in folklore or traditional system of medicine for treatment of various kinds of illness including cancer. Recently, a greater emphasis has been given towards research on complementary and alternative medicine that deals with cancer management. In traditional medicine, plants are being used for healing purposes and are effective as they contain biologically active components which are non toxic. With an understanding of

cell biology, mechanism based bioassays have become increasingly important and bio-activity guided phytochemical investigation has resulted in the isolation andcharacterization of several new molecules possessing interesting medicinal properties. Certain products from plants are known to induce apoptosis in neoplastic cells but not in normal cells.

2. Review of literature and development in the subject (Previous work done in relevant area):

It is well known fact that, the herbal medicines exist in different cultures and civilization e.g. Egyptians, Western, Chinese and Unani. Ayurveda and Chinese medicinal systems are the most acceptable traditional system which has a considerable amount of research on pharmacognocy, chemistry, pharmacology and clinical therapeutics. Ethanopharmacology and natural products provides a significant hope in drug development. Indeed many pharmacological classes of anticancer drugs available in the market are derivedfrom natural products including vincristine & vinblastine from *Catharanthus roseus,* paclitaxel (taxol) from *Taxus brevifolia,* camptothecin from *Camptotheca acuminate,* podophyllotoxin from *Podophyllum peltatum.* Most of these plants derived drugs were originally discovered through the study of traditional cures and folk knowledge.

In this view the research is focusing on the search for new types of natural chemotherapeutic agents derived from plants which are proving to be excellent sources of novel compounds. Various plants that have been established as potential agents against breast cancer such as, *Withania somnifera, Emblica officinalis, Argemone mexicana, Aegle marmelos, Morinda citrifolia, Moringa oleifera, Glycyrrhiza glabra, Nigella sativa, Rubia cordifolia, Camellia sinensis,* etc. have shown to be the cytotoxicity against cancer cells. The anti-cancer cancer activity of these plants has been associated with the inhibition of proliferation, cell growth, DNA, invasion, metastasis, angiogenesis and induction of cell cycle arrest and apoptosis in cancer cell (Tripathi et al., 2015).

3. Objectives of Research/ Proposed Hypothesis:

1. Collection, Identification, and authentication of selected medicinal plants.
2. Phytochemical extraction, separation and characterization of selected plants sample.

3. Separation of plants constituent by using column chromatography and its separation using GC-MS.
4. Evaluation of anti-cancer activity of plants by using MTT assay.
5. In-vitro morphological evaluation of apoptosis indication by Hoechst staining.
6. Evaluation of DNA damage by DNA fragmentation assay
7. Assessment of in-vivo acute toxicity of potent plant samples.

4. Methods to be adopted:

1] Extraction, isolation, purification and characterization of bioactive molecules:

(Bucar F et al., 2013)

The Soxhlet extraction system will be used for the extraction of plant. The plant extracts usually occur as a combination of various types of bioactive compounds or phytochemicals with different polarities. Therefore the isolation of these bioactive compounds will be carried out by using a number of different separation techniques such as TLC, column chromatography, flash chromatography, and HPLC to obtain the pure compounds. The pure compounds will be then used for the determination of structure and biological activities. Besides that, spectroscopic methods (Fourier-transform infrared spectroscopy (FTIR), ^{1}HNMR and Mass spectrum) will also be used to obtain and facilitate the identification of the bioactive compounds.

2] MTT cell Proliferation Assay: (Satyavani K et al., 2011)

Measurement of cell viability and proliferation forms the basis for numerous in vitro assays of a cell population's response to external factors. The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means. The MTT Cell Proliferation Assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability

3] Detection of nuclear fragmentation: (Lee SH et al., 2013)

Intracellular event of the nuclear fragmentation is a late marker of apoptosis. Apoptotic cells are stained by the addition of DNA fluorochromes (*e.g.*, acridine orange) which are able to cross the intact plasma membrane. This can be done with DAPI on fixed cells. The stained DNA allows

the altered morphology of the nuclear chromatin to be visualized by fluorescence microscopy. It is quick and inexpensive method and provides discrimination between viable and dead cells when counterstained with propidium iodide using vital dyes *e.g.,* acridine orange, Hoechst 33342.

4] Detection of apoptosis (Annexin V assay): (Rieger AM et al., 2011)

This assay is used to count the number of cells that have undergone apoptosis. Apoptosis will be detected by initially staining the cells with Annexin V and propidium iodide solution followed by flow cytometry analysis. It is based on the principle that normal cells are hydrophobic in nature as they express phosphatidyl serine in the inner membrane and when the cells undergo apoptosis, the inner membrane flips to become the outer membrane, thus exposing phosphatidyl serine. The exposed phosphatidyl serine is detected by Annexin V, and propidium iodide stains the necrotic cells, which have leaky DNA content that help to differentiate the apoptotic and necrotic cells.

5] Acute toxicity study: (Adeyemo-Salami OA et al., 2013)

Toxicity testing of innovative compounds is crucial for drug development process. The preclinical toxicity testing on various biological systems reveals the species-, organ- and dose-specific toxic effects of an investigational product. The toxicity of substances can be observed by *in vivo* exposure on experimental animals. The pre-clinical toxicity testing helps to calculate "No observed adverse effect level" which is needed to initiate the clinical evaluation of investigational products. This is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgement on the acute toxicity of the test substance.

5. Importance of study/society application:

Cancer is responsible for many deaths every year. Thus, various research groups worldwide are involved in search of safer anti-cancer agents. Apart from the use of surgery and radiotherapy, chemotherapy still remains an important option for the treatment of cancer. For the treatment of cancer, various anticancer drugs, i.e., 5-fluorouracil, cisplatin, paclitaxel, and docetaxel are available in the market. These drugs cannot be used continuously for long time as they can cause serious side effects such as neutropenia, venous thrombosis, atrial arrhythmia, mucositis, and respiratory infection. Thus, the search for novel anti-cancer agents and new approaches to cancer treatment is a highly active research field, stimulated by the discovery of new biological targets

and by the possibility of achieving newer anti-cancer agents with high efficacy and selectivity, low toxicity, and minimum side effects.

6. Proposed work Plan/ Formulation and Structure of Study:

Year-wise plan of work and targets to be achieved

Year I: Literature collection and course work, Collection and identification of plant samples, Preparation and Extraction of plant samples.

Year II: Qualitative and quantitative test of plant samples, Separation of Phytochemicals by using HPTLC/GC-MS.

Year III: Screening and evaluation of Plant extracts for anti-cancer activity by using various methods mentioned above.

Presentation of research work, publication of research work and attending conferences will be done alongside of all the activities mentioned above.

7. References:

1) Adeyemo-Salami OA, Makinde JM. Acute and sub-acute toxicity studies of the methanol extract of the leaves of Paulliniapinnata (Linn.) in Wistar albino mice and rats. Afr J Med Med Sci. 2013 Mar;42(1):81-90.
2) Bray, Freddie, Jacques Ferlay, Isabelle Soerjomataram, Rebecca L. Siegel, Lindsey A. Torre, and Ahmedin Jemal. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA-CANCER J CLIN 68, no. 6 2018;(2018):394-424.
3) Bucar F, Wube A, Schmid M. Natural product isolation--how to get from biological material to pure compounds. Nat Prod Rep. 2013 Apr;30(4):525-45.
4) Cragg, Gordon M., David J. Newman, and Stringner S. Yang. Natural product extracts of plant and marine origin having antileukemia potential. The NCI experience. J Nat Prod. 69, no. 3 (2006): 488-498.
5) Dua, R.; Shrivastava, S.; Sonwane, S.K.; Srivastava, S.K. Pharmacological Significance of Synthetic Heterocycles Scaffold : A Review. Adv. Biol. Res. (Rennes). 2011, 5, 120–144.

6) Gacche RN, Shegokar HD, Gond DS, Yang Z, Jadhav AD. Evaluation of selected flavonoids as antiangiogenic, anticancer, and radical scavenging agents: an experimental and in silico analysis. Cell Biochem Biophys. 2011;61(3):651-63.
7) Gupta RR, Kumar M, Gupta V. Heterocyclic Chemistry, Springer Publication, 1998; 1, 13-14.
8) Jonkman JE, Cathcart JA, Xu F, Bartolini ME. An introduction to the wound healing assay using live-cell microscopy. Cell AdhMigr. 2014;8(5):440-51.
9) Krishnamurthi, K. Screening of natural products for anticancer and antidiabetic properties. Health Administrator. XX (1&2) 69 (2000): 2000.
10) Lee SH, Meng XW, Flatten KS, Loegering DA. Phosphatidylserine exposure during apoptosis reflects bidirectional trafficking between plasma membrane and cytoplasm. Cell Death Differ. 2013 Jan;20(1):64-76.
11) Lokman NA, Elder AS, Ricciardelli C, Oehler MK. Chick chorioallantoic membrane (CAM) assay as an in vivo model to study the effect of newly identified molecules on ovarian cancer invasion and metastasis. Int J Mol Sci. 2012;13(8):9959-70.
12) Martins P, Jesus J, Santos S, Raposo LR, Roma-Rodrigues C, Baptista PV, FernandesAR.Heterocyclic Anticancer Compounds: Recent Advances and the Paradigm Shift towards the Use of Nanomedicine's Tool Box. Molecules. 2015 Sep 16;20(9):16852-91.
13) Niyazi M, Niyazi I, Belka C. Counting colonies of clonogenic assays by using densitometric software. RadiatOncol. 2007 Jan 9;2:4.
14) Qin, Weiwei, Guan Huang, Zuanguang Chen, and Yuanqing Zhang. Nanomaterials in targeting cancer stem cells for cancer therapy. Front Pharmacol. 2017;8 (2017):1.
15) Rieger AM, Nelson KL, Konowalchuk JD, Barreda DR. Modified annexin V/propidium iodide apoptosis assay for accurate assessment of cell death. J Vis Exp. 2011 Apr 24;(50).
16) Satyavani K, Gurudeeban S, Ramanathan T, Balasubramanian T. Biomedical potential of silver nanoparticles synthesized from calli cells of Citrulluscolocynthis (L.) Schrad. J Nanobiotechnology. 2011 Sep 26;9:43.
17) Siegel, Rebecca L., Kimberly D. Miller, and Ahmedin Jemal. Cancer statistics, 2016." CA-CANCER J CLIN. 66, no. 1 2016;(2016):7-30.
18) Stevens MFG, McCall CJ, Lelieveld P, Alexander P, Richter A, Davies DE Synthesis of polyhydroxylated 2- phenylbenzothiazoles and a comparison of their cytotoxicities and pharmacological properties with Genistein and Quercetin. J Med Chem. 1994; 37: 1689-1695.
19) Sullivan R, Holden T, Tremberger G, Cheung E, Branch C, Burrero J, et al. Fractal dimension of breast cancer cell migration in a wound healing assay. Int J Biol Life Sci. 2010;6(3):170-175.

20) Tripathi P, Singh A. Indigenous Asian Plants against Cancer: A Comprehensive Review. Int J Plant Res. 2015;5(4):80-86.
21) Wheeler RJ. Analyzing the dynamics of cell cycle processes from fixed samples through ergodic principles. MolBiol Cell. 2015 Nov 5;26(22):3898-903.

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